

SEQUENCE LISTING

<110> RIKEN

<120> A MUTANT KANAMYCIN NUCLEOTIDYLTRANSFERASE AND A METHOD OF
SCREENING THERMOPHILIC BACTERIA USING THE SAME

<130> PH-1082

<140>

<141>

<150> JP 11-309616

<151> 29-OCT-1999

<160> 11

<170> PatentIn Ver. 2.0

<210> 1

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

<400> 1

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Tyr
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
85 90 95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
245 250

<210> 2

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant enzyme obtained by introduction of point mutation
into wild type KNT gene of Staphylococcus aureus and its expression

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Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe
50 55 60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp
85 90 95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro
100 105 110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu
195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
245 250

<210> 3

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant enzyme obtained by introduction of point mutation into wild type KNT gene of Staphylococcus aureus and its expression

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Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
180 185 190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Pro Gly Tyr Val Gln Leu
195 200 205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
245 250

<210> 4

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:5'-Primer for PCR
amplification

<400> 4

<210> 5

<211> 35

<212> DNA

<213> Artificial Sequence

$\langle 220 \rangle$

<223> Description of Artificial Sequence: 3'-Primer for PCR amplification

<400> 5

gactgtacgc tgcagcgtaa ccaacatgat taaca

35

<210> 6

<211> 35

<212> DNA

<213> Artificial Sequence

 $\langle 220 \rangle$

<223> Description of Artificial Sequence: 5'-Primer for PCR amplification

<400> 6

gactgtacgg aattcgagct cgagcaaatc taaaa

35

<210> 7

<211> 35

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:5'-Primer for subcloning of
WT*

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<210> 8

<211> 35

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:5'-Primer for subcloning of
KT3-11 and HTK

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gactgtacgc atatgaaagg accaataata atgac

35

<210> 9

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:3'-Primer for subcloning

<400> 9

35

gactgtacgc tcgagcgtaa ccaacatgat taaca

<210> 10

<211> 759

<212> DNA

<213> Staphylococcus aureus

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Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

1

5

10

15

cat gaa att aag gaa cga ata ttg gat aaa tat ggg gat gat gtt aag 96
His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys

20

25

30

gct att ggt gtt tat ggc tct ctt ggt cgt cag act gat ggg ccc tat 144
Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr

35

40

45

tcg gat att gag atg atg tgt gtc atg tca aca gag gaa gca gag ttc 192
Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe

50

55

60

agc cat gaa tgg aca acc ggt gag tgg aag gtg gaa gtg aat ttt gat 240
 Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp
 65 70 75 80

agc gaa gag att cta cta gat tat gca tct cag gtg gaa tca gat tgg 288
 Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
 85 90 95

ccg ctt aca cat ggt caa ttt ttc tct att ttg ccg att tat gat tca 336
 Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
 100 105 110

ggt gga tac tta gag aaa gtg tat caa act gct aaa tcg gta gaa gcc 384
 Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
 115 120 125

caa acg ttc cac gat gcg att tgt gcc ctt atc gta gaa gag ctg ttt 432
 Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
 130 135 140

gaa tat gca ggc aaa tgg cgt aat att cgt gtg caa gga ccg aca aca 480
 Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
 145 150 155 160

ttt cta cca tcc ttg act gta cag gta gca atg gca ggt gcc atg ttg 528
 Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
 165 170 175

att ggt ctg cat cat cgc atc tgt tat acg acg agc gct tcg gtc tta 576
 Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
 180 185 190

act gaa gca gtt aag caa tca gat ctt cct tca ggt tat gac cat ctg 624
 Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
 195 200 205

tgc cag ttc gta atg tct ggt caa ctt tcc gac tct gag aaa ctt ctg 672
 Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
 210 215 220

gaa tcg cta gag aat ttc tgg aat ggg att cag gag tgg aca gaa cga 720
 Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
 225 230 235 240

cac gga tat ata gtg gat gtg tca aaa cgc ata cca ttt 759
 His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
 245 250

<210> 11

<211> 253

<212> PRT

<213> Staphylococcus aureus

<400> 11

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val
 1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe
50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp
85 90 95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser
100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala
115 120 125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu
165 170 175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu
180 185 190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu
195 200 205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu
210 215 220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg
225 230 235 240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe
245 250